REMARKS

Claim Amendments

Claims 9-13, 18-22, 24-37 and 41, directed to non-elected inventions, have been cancelled, without prejudice to or disclaimer of the subject matter therein. Applicants expressly reserve the right to pursue the subject matter of any non-elected claims in a divisional application without the need to file a terminal disclaimer.

Claims 23 and 42-45 are currently maintained as Withdrawn, pursuant to Applicants intent to request rejoinder of any method claims with allowable product claims.

New Claims 46-57 are based on specific embodiments of original claims from which they depend, or are otherwise supported in the specification on page 29, lines 13-24.

Objections to the Specification

The Examiner has objected to the specification. First, on page 11, the Examiner contends that the description of Figure 1 does not match the drawings.

In reply, although Applicants believe that the prior language was sufficiently descriptive of the figures, since Figs. 1A and 1B do show digital images showing prelamin A GFP fusion protein expression and processing, this sentence has been rewritten in the form suggested by the Examiner.

Second, the Examiner contends that on page 79, line 18, the beginning of the sentence is not capitalized.

This sentence has been amended to capitalize the first letter of the sentence.

As a result of the Examiner's notation of a typographical error in Claim 40, Applicants have noted that the same error appears in some portions of the specification. These have been corrected and the correct mutation is shown by reference to SEQ ID NO:4 and page 75, line 6.

In view of the foregoing amendments and remarks, the Examiner is respectfully requested to withdraw the objections to the specification.

Objections to the Claims

The Examiner has objected to Claim 39 as not being uniform in the identification of amino acids. The Examiner suggests that all amino acids be referenced using the three letter abbreviation.

Claim 39 has been amended to adopt the Examiner's suggested method for referencing amino acids.

The Examiner has objected to Claim 40 for containing a typographical error in that "Asn19Lys" should be "Asn195Lys".

Claim 40 has been amended to correct the typographical error.

In view of the foregoing amendments and remarks, the Examiner is respectfully requested to withdraw the objections to the claims.

Objection to the Specification and Rejection of Claims 1-8, 14-16 and 38-40 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 1-8, 14-16 and 38-40 under 35 U.S.C. § 112, first paragraph, on the basis of enablement. Specifically, the Examiner contends that the specification is enabling for a composition comprising SEQ ID NO:4 that affects the formation of normal nuclear lamina structures and the differentiation of cardiac and skeletal myoblasts, but is not enabling for isolated complexes comprising fragments, peptides that differ by at least one substitution, deletion, or insertion, or peptides that are at least 70% identical to SEQ ID NO:2 or SEQ ID NO:4. The Examiner asserts that there is precedent in the art that protein farnesyltransferase and geranylgeranyltransferase enzymes have binding sites that require distinct tertiary structures for proper substrate binding, and contends that there is no way to predict whether any of the claimed fragments or homologues will interact with these enzymes. The Examiner acknowledges that the specification provides guidance with respect to the site-directed mutagenesis of SEQ ID NO:4 and the affect on myotube formation, but asserts that there is no guidance with respect to selecting fragments and homologues that might affect nucelar lamina function on developing myotubes, or how to determine which fragments or homologues might work.

Applicants traverse the Examiner's rejection of Claims 1-8, 14-16 and 38-40 under 35 U.S.C. § 112, first paragraph. First, Applicants submit that at the time of the invention, information was

available regarding the series of post-translational protein modifications that result in the processing of prelamin A into lamin A, including the importance of the CAAX motif and its farnesylation (see specification page 19, lines 9-18). Therefore, at the time of the invention, one was already apprised of where the prelamin A protein is likely or not likely to tolerate modifications, based on the requirement for prenylation and endoprotease processing of prelamin A in order to release lamin from prelamin A. The present inventor has, for the first time, discovered the functional *significance* of this processing step, including the discovery of a biological function for the prepeptide and the specific role of prelamin A processing in regulating muscle cell differentiation. With this discovery, one of skill n the art can now make and use the prelamin A prepeptide, a prelamin A protein, and a processing-deficient prelamin A protein as described by the present invention, making use of the structural knowledge that was already available in the art for this protein.

Second, the Examiner presents an argument that the art has shown that protein farnesyltransferase and geranylgeranyltransferase enzymes have binding sites that require distinct tertiary structures for proper substrate binding and therefore, that there is no way to predict the effect of modifications on these enzymes. The Examiner refers to Park et al. and states that this reference teaches that the affinity of a protein substrate for farnesyltransferase (FT) is affected by basic amino acid residues upstream of the CAAX motif, which encompass amino acids disclosed by SEQ ID NO:2 and SEQ ID NO:4. However, Applicants submit that Park et al. simply teach that the affinity of a protein substrate for FTase is *enhanced* by the presence of multiple basic residues just upstream of the CAAX region - Park et al. do not teach that such a region is necessary or critical for substrate binding as the Examiner seems to imply (note that H-Ras, lacking such a polybasic region, is taught to bind the FTase by Park et al. (see page 1804)). Moreover, if one reviews the sequence of SEQ ID NO:4 (Prelamin A), which is known to be farnesylated, there do not appear to be multiple basic amino acid residues just upstream of the CAAX motif (positions 661-664 of SEQ ID NO:4). Indeed, the only basic amino acid of SEQ ID NOs:2 and 4 that is just upstream of the CAAX motif is position 654 of SEQ ID NO:4 (position 8 of SEQ ID NO:2), which the specification clearly teaches is a weak position (*i.e.,* not recommended) for modification (see page 36, lines 5-6) in any event. Therefore, the teachings of Park et al. regarding the polybasic region seem only to indicate that

Prelamin A is likely to have weaker affinity for an FTase than a protein with a polybasic region. Applicants submit that this teaching in Park et al. does not indicate that one of skill in the art would not be able to modify the claimed proteins without undue experimentation.

Indeed, rather than providing evidence that it is unpredictable how to modify a farnesylated protein as the Examiner asserts, to the contrary, Applicants submit that the teachings of Park et al., which provide the crystal structure of an FTase enzyme bound to a peptide having a CAAX motif and discusses in detail the interactions that are believed to mimic normal CAAX peptide binding, in fact provide one of skill in the art with additional knowledge that can be used in consideration of where to modify a protein substrate for FTase. Such a teaching only *enhances* the present specification disclosure, which provides a detailed description (discussed below) of what residues in SEQ ID NO:2 and SEQ ID NO:4 may be modified to preserve or disrupt activity of the proteins.

Furthermore, with regard to SEQ ID NO:2, Applicants submit that one can predictably prenylate this peptide and produce a biologically active peptide, even in the absence of the tertiary structure or additional upstream amino acids that are present in the Prelamin A protein. As evidence of this point, the attached publications by Xie et al. and Sherrill et al. show that the yeast a-type mating pheromone, to which the present inventor has demonstrated the prepeptide of Prelamin A is functionally analogous, can be synthetically produced and is readily farnesylated as a biologically active peptide. Moreover, the references of Xie et al. and Sherrill et al. provide significant information regarding the impact of the farnesyl group on biological activity of the peptide, and show how the farnesyl moiety of the yeast a-type mating pheromone can be modified to decrease or retain biological activity.

With more particular regard to Claims 1-8, which are directed to the prepeptide of prelamin A (SEQ ID NO:2), Applicants submit that the Examiner is incorrect in stating that the specification provides no guidance with respect to selecting fragments and homologues that might affect nuclear lamina formation or myotubes, or how to determine which fragments and homologues might work. To the contrary, the specification, at page 34, line 17 to page 37, line 2, provides a <u>detailed</u> discussion of which of *each* of the positions of the 15 amino acid sequence of SEQ ID NO:2 are candidates for modification and which are not. The discussion includes the consideration of

conservation or variation of the amino acids across species, and further teaches that the C-terminal cysteine residue in SEQ ID NO:2 (position 15 of SEQ ID NO:20 and position 661 of SEQ ID NO:4) is the residue targeted for farnesylation (noting that this residue is not targeted for modification in the teachings of the specification). Moreover, the discussion includes specific recommendations for the type of substitution that may be made at each position of SEQ ID NO:2 where it is likely that a substitution can be introduced. The specification also shows the structural relationship of the prepeptide sequence and processing site among five different animal species (see Fig. 2), providing further evidence as to which amino acid positions can predictably tolerate modification while retaining function. The present inventor teaches that the prelamin A prepeptide functions as a signaling molecule when proteolytically released from the prelamin A protein, indicating the proximity and direction of mononucleate myoblasts during differentiation and cell fusion to generate multinucleate myocytes, and given the guidance provided in the specification, one of skill in the art would readily be able to determine whether a homologue has the required activity. The specification provides assays (see Examples) that one can use to confirm the activity, which contradicts the Examiner's assertion that the specification does not provide guidance to determine which fragments and homologues might work.

It is further noted that the recitation of homologues with at least 70% identity to SEQ ID NO:2 allow homologues with only up to four modifications, recitation of homologues with at least 80% identity to SEQ ID NO:2 allow homologues with only up to three modifications, recitation of homologues with at least 85% identity to SEQ ID NO:2 allow homologues with only up to two modifications (see new Claim 46), and recitation of homologues with at least 90% identity to SEQ ID NO:2 allow homologues with only one modification. Therefore, the number of possible homologues, particularly given the guidance provided in the specification regarding where modifications are likely to be tolerated, is not large. Referring to Fig. 2, one can see that the mouse and human prepeptide sequences differ by two amino acids, and the mouse prepeptide represents a functional prelamin A prepeptide. Comparing the human prepeptide to that of chicken, Xenopus and zebrafish, one can see that the sequences are outside the scope of what is claimed (*i.e.,* these functional sequences differ from SEQ ID NO:2 by more than four modifications, which is the 70%

identity level), thus further indicating the predictability of modifying SEQ ID NO:2 within the claimed scope while retaining function of the prepeptide. Applicants note that the Examiner has rejected Claim 6, which recites a protein consisting essentially of SEQ ID NO:2. It is unclear why the Examiner considers this claim to lack enablement given that the Examiner's arguments have been directed to homologues of the recited sequences.

Moreover, given the guidance provided in the specification discussed above regarding likely positions of SEQ ID NO:2 for modification, the number of residues that one would delete to form a fragment would be limited, such that one of skill in the art could readily make and use functional fragments of SEQ ID NO:2 (*e.g.,* one would not truncate the protein at position 15, and only positions 1 and 2 are recommended for modification by the specification). Finally, with regard to Claim 1 part (d), the specification specifically addresses why these positions are candidates for modification, and teaches what residues may be used in certain of the positions.

With regard to Claims 14-16 and 38-40, Applicants submit that the present specification also provides guidance regarding what residues of prelamin A (SEQ ID NO:4) are predicted to tolerate modification or, with regard to Claims 38-40, will produce a processing deficient prelamin A protein. Initially, Applicants submit that the discussion above with regard to SEQ ID NO:2 (referring also to the discussion of SEQ ID NO:20 and Fig. 2) can also be applied to modification of SEQ ID NO:4, since prelamin A processing occurs in this region. In addition, the specification provides evidence that at least six different mutations in prelamin A are asserting their effect by negatively impacting prelamin A processing, thus providing information regarding residues that can be modified to produce a processing-deficient prelamin A or should be avoided in order to produce a functional prelamin A. Furthermore, at the time of the invention, the nucleic acid and amino acid sequence of prelamin A were known for a variety of animal species, including, but not limited to: human mouse, chicken, *Xenopus laevis* (African clawed frog), and *Danio rerio* (zebra fish). These are all provided by the present specification, and one of skill in the art can readily determine which residues are likely to tolerate modification and make and use the claimed modified proteins without undue experimentation.

With regard to Claims 39 and 40, the specific positions recited in Claim 39 and 40 have been clearly identified by the present specification as likely to negatively affect prelamin A processing or have actually been demonstrated to negatively affect prelamin A processing if modified. Therefore, it is unclear how the Examiner considers these claims to lack enablement. For example, modifications at Arg60, Leu85, Glu203, Arg89, Asn195, and Arg377 are shown to negatively impact prelamin A processing and resultant myotube formation and myoblast differentiation. Indeed, the Examples of the present specification show that the specific mutations recited in Claim 40 result in processing-deficient prelamin A proteins. The other recited residues recited in Claim 39 near the CAAX processing site, and particularly the cysteine at position 661 of SEQ ID NO:4, could clearly be modified to disrupt prelamin A processing, especially given the guidance provided in the specification regarding these positions, and the knowledge in the art regarding the requirements for prelamin A endoprotease processing. Moreover, it is believed that the present specification has demonstrated that prelamin A processing regulates the activities recited in Claim 38, upon which these claims depend.

In summary, Applicants submit that the present specification provides sufficient guidance to make and use the claimed proteins, including the claimed homologues and fragments. In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1-8, 14-16 and 38-40 under 35 U.S.C. § 112, first paragraph.

Objection to the Specification and Rejection of Claims 1-5, 7-8, 14-16, 38 and 39 Under 35 U.S.C. § 112, First Paragraph:

The Examiner has objected to the specification and rejected Claims 1-5, 7-8, 14-16, 38 and 39 under 35 U.S.C. § 112, first paragraph, on the basis of written description. Specifically, the Examiner contends that the specification does not describe the amino acids in the various polypeptides that can be altered without affecting the function of the specific polypeptide. The Examiner asserts that the claims encompass peptides that could have more than one deletion, including deletion of all of the enumerated positions. With regard to substitutions, the Examiner

contends that the specification does not describe the effects of the altered peptide substrates on enzyme-substrate association.

Applicants traverse the rejection of Claims 1-5, 7-8, 14-16, 38 and 39 under 35 U.S.C. § 112, first paragraph. Initially, Applicants refer to the discussion above, and submit that contrary to the Examiner's contention, the specification provides detailed guidance regarding which amino acids in the polypeptides can be altered without affecting the function of the specific polypeptide. With regard to Claims 38 and 39, it is noted that the goal *is* to affect the function of the polypeptide (*i.e.*, by creating a processing-deficient prelamin A protein).

Initially, with regard to deletions and substitutions, it is noted that Claims 1-8 have been amended in part (d) so that the deletions or substitutions do not include all of the enumerated positions. With further regard to Claims 1-8, as discussed in detail above, the present specification provides significant guidance at page 34, line 17 to page 37, line 2, with respect to which of *each* of the positions of the 15 amino acid sequence of SEQ ID NO:2 are candidates for modification and which are not. Moreover, the number of variants encompassed by these claims is not large, noting that at 70% identity, only 4 or fewer amino acids can be modified. With regard to deletion fragments, given the discussion with regard to which positions are likely to tolerate modification, it is clear that such fragments are limited, with deletions at positions 1 and 2 being the only likely candidates. The Examiner contends that the specification does not describe the effects of altered peptide substrates on enzyme-substrate association, but Applicants submit that this is incorrect. The specification clearly discusses the impact of modification at each position and clearly notes those residues that are important for function (*e.g.*, the cysteine residue at position 15 of SEQ ID NO:2). With regard to Park et al., as discussed above, Applicants have discussed above that the Examiner's reference to the polybasic region is not applicable to this protein and further, using the modified yeast a-type mating pheromone as a reference, one of skill in the art can readily produce and farnesylate the peptides encompassed by Claims 1-8, noting that the farnesylation occurs on the position 15 cysteine, which the present specification teaches should be retained.

With regard to Claims 14-16 and 38-39, Applicants submit that one of skill in the art can also readily envision the proteins encompassed by these claims. The specification and the knowledge in

the art at the time of the invention provide guidance regarding the processing site for prelamin A, as well as the residues of prelamin A that are rarely modified across species or vary (referring to discussions at page 18, line 17 to page 19, line 18; page 34, lines 17-25; and page 37, lines 10-19, for example). The specification has taught which residues are most important to the function of prelamin A function by noting residues that are important with respect to the processing site and by identifying residues that, if modified, can render the prelamin A processing-deficient. Again, with regard to Park et al., Applicants submit that this reference only *assists* one of skill in the art with predictably modifying a substrate for an FTase, as discussed above. One of skill in the art is able to envision proteins that are 70%, 95%, 97% or 99% identical to SEQ ID NO:4 (referring also to newly added claims), and the specification provides assays that can be used to evaluate the function of the proteins. With regard to Claim 38, the specification provides a specific identification of several residues that could be targeted to disrupt prelamin A processing, and further provides working examples illustrating that several of these positions result in a processing-deficient prelamin A protein. With regard to Claim 39, the Examiner's rejection is completely unclear, since this claim specifically recites sites for modification in prelamin A, several of which are shown by the specification to affect prelamin A processing and the functions associated with this processing that have been discovered by the present inventor.

In view of the foregoing amendments and remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1-5, 7-8, 14-16, 38 and 39 under 35 U.S.C. § 112, first paragraph.

Rejection of Claims 1-8 Under 35 U.S.C. § 102(b):

The Examiner has rejected Claims 1-8 under 35 U.S.C. § 102(b), contending that these claims are anticipated by Kilic et al. The Examiner asserts that Kilic et al. disclose the solid phase synthesis of a peptide that has the sequence H_2N-RSYLLGNSSPRTQSPQNC-OCH_3. Therefore, the Examiner contends that this peptide has 100% identity with SEQ ID NO:2 plus the insertion of three residues "RSY" at the amino terminus.

Applicants traverse the rejection of Claims 1-8 under 35 U.S.C. § 102(b). Applicants initially refer the Examiner to page 40, line 20 to page 41, line 12 of the instant specification, where the phrase "consisting essentially of" is defined. According to the present specification, a sequence consisting essentially of a specified sequence (*e.g.*, SEQ ID NO:2) has "at least one, and up to about 20, additional heterologous amino acids flanking each of the C- and/or N-terminal ends of the specified amino acid sequence." The specification goes on to define"heterologous amino acids" as "amino acids that are not naturally found (*i.e.*, not found in nature, *in vivo*) flanking the specified amino acid sequence, or that are not related to the function of the specified amino acid sequence, or that would not be encoded by the nucleotides that flank the naturally occurring nucleic acid sequence encoding the specified amino acid sequence as it occurs in the gene, if such nucleotides in the naturally occurring sequence were translated using standard codon usage for the organism from which the given amino acid sequence is derived".

Accordingly, the peptide disclosed by Kilic et al. fails to anticipate the presently claimed invention. More specifically, the inclusion of "RSY" to the peptide of Kilic et al. represents the addition of the *actual* amino acids that occur next to the Pre sequence of SEQ ID NO:2 in nature, and since Kilic et al. do in fact confirm that the "RSYLLG" sequence is the natural endoprotease cleavage site in prelamin A across species (see Table 1 of Kilic et al.), then the peptide of Kilic et al. does not meet the limitation of a peptide consisting essentially of SEQ ID NO:2.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 1-8 under 35 U.S.C. § 102(b).

Rejection of Claims 38-40 Under 35 U.S.C. § 102(b):

The Examiner has rejected Claims 38-40 under 35 U.S.C. § 102(b), contending that these claims are anticipated by Fatkin et al. The Examiner contends that Fatkin et al. disclose four processing deficient prelamin A peptides, wherein the peptides consist of amino acid sequences that differ from SEQ ID NO:4 by a substitution of an amino acid in SEQ ID NO:4. The Examiner further states that the disclosed substitutions are Arg60Gly, Leu85Arg, Asn195Lys, and Glu203Gly.

Applicants traverse the rejection of Claims 38-40 under 35 U.S.C. § 102(b). Contrary to the Examiner's contention that Fatkin et al. disclose four processing deficient prelamin A peptides, Applicants submit that Fatkin et al. only identified four different mutations in the *genes* of members of two families with autosomal dominant dilated cardiomyopathy and conduction-system disease. Fatkin et al. do not actually produce or isolate any proteins, nor do Fatkin et al. teach or suggest that one should produce or isolate the proteins. Therefore, Fatkin et al. do not teach an isolated protein consisting essentially of a protein that differs from SEQ ID NO:4 (prelamin A) by at least one substitution, deletion or insertion that results in a decrease in a prelamin A or prelamin A pre peptide biological activity that is recited in the claims. Moreover, Fatkin et al. do not appreciate the actual functional effect of the mutations on prelamin A (*i.e.*, they do not know that the mutations will lead to a processing-deficient prelamin A and therefore do not teach or suggest that the processing-deficient prelamin A will lead to decrease any of the recited biological activites discovered by the present inventor). Therefore, Applicants submit that Fatkin et al. could not even suggest the production of a protein as presently claimed, because absent such an appreciation with regard to the utility of producing such proteins as disclosed in the present application, there would be no reason that Fatkin et al. would suggest producing or isolating the proteins.

In view of the foregoing remarks, Applicants respectfully request that the Examiner withdraw the rejection of Claims 38-40 under 35 U.S.C. § 102(b).

Applicants have attempted to respond to all of the Examiner's concerns as set forth in the October 19 Office Action. Any questions or concerns regarding the claims or Applicants' position should be directed to the below-named agent at (303) 863-9700.

Respectfully submitted,

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